ISSN 1346-7565

Acta Phytotax. Geobot. 68 (1): 17–22 (2017)
doi: 10.18942/apg.201615

A *Cladopus* Species (Podostemaceae) Rediscovered from Hong Kong

Masahiro Kato[1,*], Mandy Chuk-Kwan Wong[2], Janice Pei-Lai Lo[2] and Satoshi Koi[3]

*[1] Department of Botany, National Museum of Nature and Science, Tsukuba 305-0005, Japan * sorang@kahaku.go.jp (author for correspondence); [2] Ho Koon Nature Education cum Astronomical Centre, 101 Route Twist, Tsuen Wan, Hong Kong, China; [3] Botanical Gardens, Osaka City University, Kisaichi, Katano, Osaka 576-0004, Japan*

A species of Podostemaceae discovered in 1963 in Hong Kong was identified as *Cladopus nymanii* s.l. or *C. austrosinensis*. No additional specimens were subsequently collected. Consequently, the identification has remained uncertain and it was thought that the plants may have been extirpated in Hong Kong. Half a century later, a species of *Cladopus* was discovered in a semi natural habitat. Comparison of the morphology and a molecular phylogenetic analysis indicated that it was *Cladopus fukienensis*. It remains to be ascertained whether it also grows in natural habitats, and if it is the only species of *Cladopus* in Hong Kong.

Key words: *Cladopus austrosinensis*, *Cladopus fukienensis*, Hong Kong, *matK* phylogeny, Podostemaceae

Podostemaceae (colloquially river-weeds) are unusual aquatic angiosperms that grow on submerged rocks in rapids and waterfalls (van Steenis 1981). The family is distributed primarily in the tropics and subtropics (Kato 2013, 2016). Southern and southeastern China, southwestern Japan, and southeastern Canada are at the northernmost limits of its distribution.

The Podostemaceae of China were first investigated by Chao (1948), who described *Lawiella chinensis* H.C. Chao, *L. fukienensis* H.C. Chao (as '*fukiensis*'), and *Terniopsis sessilis* H.C. Chao. Since then, *Cladopus* (Chun & Tsiang 1963) and *Hydrobryum* (Tao 1983) have been added, *Terniopsis* was redescribed (Chao 1980), and the two species of *Lawiella* were combined with *Cladopus* H. A. Möller (Chao 1982). Wu (1988) classified the family into three genera and three species in China; *Terniopsis sessilis* in Fujian, *Hydrobryum griffithii* Tul. in Yunnan, *Cladopus nymanii* H. A. Möller sensu lato [including *C. fukienensis* (H.C. Chao) H.C. Chao and *C. chinensis* (H.C. Chao) H.C. Chao, and *C. japonicus* Imamura] in Fujian, Guangdong and Hainan. Qiu *et al.* (2003) recognized three genera and four species in China; *Dalzellia sessilis* (C.H. Chao) C. Cusset & G. Cusset, *Hydrobryum griffithii*, *Cladopus nymanii* s.l., and *C. chinensis*. Kato & Kita (2003) recognized three genera and six species in China, *Terniopsis sessilis*, *Hydrobryum griffithii*, *H. japonicum* Imamura from Yunnan, *Cladopus austrosinensis* M. Kato & Y. Kita from Guangdong and Hainan, *C. fukienensis* and *C. japonicus* including *C. chinensis* [= *C. doianus* (Koidz.) Koriba (Kato 2008)].

In 1963, the Japanese bryologist Zennoske Iwatsuki collected the first specimen of *Cladopus* (*Z. Iwatsuki 63* [TI]) in Hong Kong on Tai Mo Shan, Kowloon Peninsula. It was identified as *C. nymanii* s.l. by Cusset (1992) and later as *C. austrosinensis* by Kato & Kita (2003) based on the few bracts on a short floriferous shoot. Because the specimen comprises dried fragments, its identity remains uncertain. Kato & Kita's (2003) treatment is followed in the Flora of Hong Kong (Deng & Xia 2008). Additional specimens have

not been gathered since the original collection, raising doubt about its continued existence in Hong Kong.

Cladopus is recognized (see Fig. 1) having subcylindrical or ribbon-like roots with tufts of leaves and flowering shoots near the margin at the sinuses of the root branches. It adheres to submerged rock surfaces and spreads vegetatively. The flowering shoot comprises digitate or lobed (in *C. taiensis* C. Cusset) bracts in two rows subtending a terminal flower. The flower bud is enclosed by a spathella (special envelope), which ruptures apically at anthesis. The simple flower comprises a pedicel, two tepals, usually one stamen and one pistil. The tepals are borne on either side of the filament and both the tepals and stamen are on the surface facing the rock. After fertilization, the ovary develops into a smooth, globose capsule on a stalk derived from the pedicel and the axis of the flowering shoot. The diagnostic characteristics for species identification include the width of the root, the length of the flowering shoot, the number and form of the bracts and the number of stamens (Cusset 1992, Kato 2006, Koi & Kato 2012).

On 27 March, 2013, Mandy Wong and colleagues discovered two populations of Podostemaceae at the foot of Tai Mo Shan, Kowloon Peninsula, Hong Kong. One of the two plants was collected from a small population in a small rapid on an open south-facing slope beside a catchwater at the foot of Tai Mo Shan. The other was collected from a smaller population on a cement wall of the same catchwater separate from the rapid. Thus they grow in semi natural and artificial habitats. The plants were preliminarily identified as a

FIG. 1. *Cladopus fukienensis*. Hong Kong population where *HongKong SN* was collected. A. Ribbon-like branched roots on rock surface. B. Clustered leaves. C. Dense flowering shoots with digitate bracts in natural habitat on rock surface. D. Flowering shoot with bracts reduced basipetally. Photographed by Mandy Wong.

species of *Cladopus* because they had diagnostic characters of *Cladopus* (Fig. 1). In this study we investigated the phylogenetic position and morphological characters of the plants.

Materials and Methods

The morphological analysis was based on plants collected recently (Table 1) and specimens of *Cladopus* from China deposited in the National Museum of Nature and Science Herbarium (TNS) and the University of Tokyo Herbarium (TI) (Kato & Kita 2003). The phylogenetic analysis of the *matK* region was based on materials cited in Table 1 and on other species of *Cladopus* and *Paracladopus* examined by Koi *et al*. (2012) (voucher IDs are shown in Fig. 2). Preliminary phylogenetic analysis using *HongKong SN* suggested that the Hong Kong plants are assignable to a clade of *C. austrosinensis* and *C. fukienensis.*

TABLE 1. Materials recently collected and examined.

Species	Locality and voucher (herbarium acronym)	GenBank accession number of *matK* sequence
Cladopus austrosinensis	Huizhou, Guangdong, China; *Kokubugata GK17125* (TNS)	LC144911
Cladopus fukienensis	Small rapid at southern foot of Tai Mo Shan, Hong Kong; *Kato et al. HongKong SN* (TNS)	LC144912
	Cement wall of catchwater at southern foot of Tai Mo Shan, Hong Kong; *Kato et al. HongKong HK2* (TNS)	LC144913

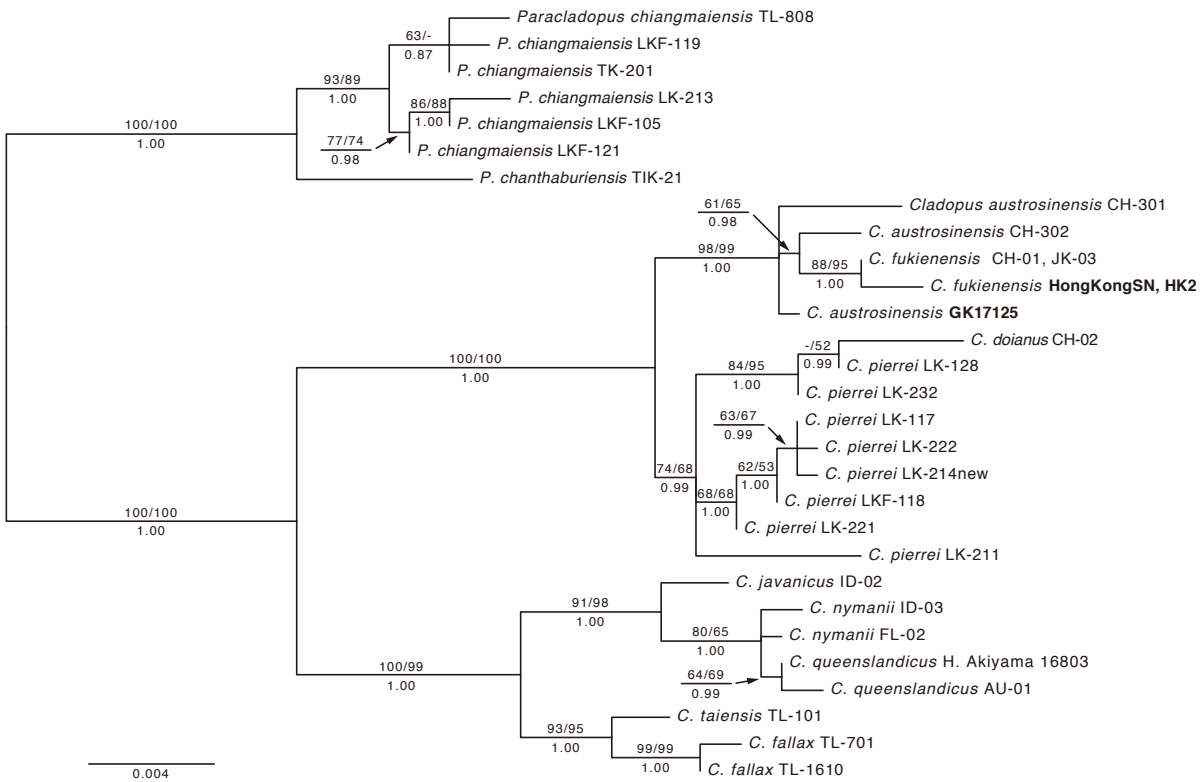

FIG. 2. Maximum Likelihood *matK* tree (-lnL = 3161.77996) of *Cladopus*. Figures on left and right sides above horizontal bars indicate Maximum Parsimony and Maximum Likelihood bootstrap values (%), respectively, those below bars indicate Bayesian posterior probabilities. *Paracladopus chanthaburiensis* and *P. chiangmaiensis* are outgroups.

For molecular phylogenetic analysis, the materials were dried with silica gel. Sequencing was conducted as previously described by Koi *et al*. (2012). The methods for constructing the phylogenetic tree followed Koi & Kato (2010) with some modifications as follows: in Maximum Likelihood (ML) analysis, the GTR+I+G model was selected for our data set: base frequencies were A = 0.3239, C = 0.1534, G = 0.1368, T = 0.3858; the substitution rate matrix was A to C = 1.4783, A to G = 0.9457, A to T = 0.2859, C to G = 0.4047, C to T = 0.9479, G to T = 1.0000; the proportion of invariable sites was 0.6890; and the gamma distribution shape parameter was 1.1780. In Bayesian (Bayes) analysis, the GTR+I+G model was used. Markov chain Monte Carlo iterations with four chains were conducted for 2000000 generations, and the first 5000 trees were discarded as burn-in. The remaining 15000 trees, whose standard deviation of split frequency was under 0.01, were used. In maximum parsimony (MP) analyses, all characters were equally weighted, and bootstrap values were calculated with heuristic searches for 1000 replicates with 100 random addition replicates with tree-bisection reconnection (TBR) branch swapping. *Paracladopus chanthaburiensis* and *P. chiangmaiensis* were used as outgroups, because *Paracladopus* is sister to *Cladopus* (Koi *et al*. 2008, 2012, Koi & Kato 2012).

Results

The Hong Kong plants, *HongKong SN* and *HongKong HK2*, had the same *matK* gene sequence. In an ML phylogenetic tree of the *matK* gene, the Hong Kong plants were sister to *C. fukienensis (CH-01)*, which all merged in the *C. austrosinensis* clade (*CH-301, CH-302, GK17125*) (Fig. 2). *CH-01* had the same sequence as *JK-03* from the Okawa River, the type locality of *C. austro-osumiensis* Kadono & N. Usui, a synonym of *C. fukienensis*, Kyushu, Japan (Kita & Kato 2004, Koi *et al*. 2012). The clade was sister to a clade consisting of *C. pierrei* and *C. doianus*, the former species of which is in Laos, Thailand and Vietnam, and the latter in Fujian and Japan.

The results of a comparison of the morphology of the Hong Kong plants, *C. austrosinensis* and *C. fukienensis* are shown in Table 2. The Hong Kong plants (*HongKong SN* and *HK2*) shared all their characters with *C. fukienensis*. Among others, the shared many basipetally reduced bracts on the long reproductive shoot are unique (Fig. 1D). Because most of the diagnostic characters of *Z. Iwatsuki 63* could not be ascertained, it was difficult to identify it as either *C. austrosinensis* or *C. fukienensis*.

Discussion

The results of the molecular phylogenetic analysis and comparative morphology indicate that *HongKong SN* and *HongKong HK2* are *C. fukienensis.* Cusset (1992) identified *Z. Iwatsuki 63* as *C. nymanii* s.l., which includes *C. doianus* [= *C. japonicus*, *C. chinensis* and *C. austrosatsumensis* (Koidz.) Ohwi] and *C. fukienensis*. However, *C. nymanii* sensu Cusset is polymorphic (Kita & Kato 2004, Koi *et al*. 2012). Kato & Kita (2003) identified *Z. Iwatsuki 63* as *C. austrosinensis*. Our study using additional specimens found that the difference between *C. fukienensis* and *C. austrosinensis* was less than given in the descriptions by Chao (1948) and Kato & Kita (2003). Nonetheless, the two species are distinguished by the length of the flowering shoots and the morphology of the bracts (Table 2). Because no molecular data are available for *Z. Iwatsuki 63*, it is not possible to identify it with certainty, but it does not exclude the possibility that *HongKong SN*, *HongKong HK2* and *Z. Iwatsuki 63* are conspecific and only *C. fukienensis* occurs in Hong Kong.

Z. Iwatsuki 63 was collected in a natural habitat at 400–500 m elevation on Tai Mo Shan, but the location is imprecisely known. In comparison, *HongKong SN* and *HongKong HK2* were from small populations beside a catchwater in semi natural habitats. It is uncertain whether *Cladopus* still occurs or has been extirpated from natural habitats.

The molecular data and comparative morphology show that *GK17125* from Huizhou,

Guangdong, is *C. austrosinensis*, thereby providing a more precise knowledge of the distribution of *C. austrosinensis* than in Kato & Kita (2003). Hong Kong is about 150 km south of the location of *C. austrosinensis* in Huizhou, but isolated from *C. fukienensis* in Fujian by a greater distance. Phylogenetically, *C. austrosinensis* is perhaps paraphyletic with *C. fukienensis* (Koi et al. 2012, present study). Further investigation in Guangdong and Fujian is necessary to clarify their distribution and specific differences.

We thank Chi-Tai Kwok who supported us in various ways, Ray Lok-Hin So for his help in the field, G. Kokubu-

TABLE 2. Comparison of characters in Hong Kong plants, *C. fukienensis* and *C. austrosinensis.*

Character	*HongKong SN, HK2* (Hong Kong)	*Z. Iwatsuki 63*[*1] (Hong Kong)	*C. fukienensis*[*2]	*C. austrosinensis*[*3]
Root	ribbon-like, (0.7–)1–2(–2.5) mm wide, 0.3–0.5 mm thick (Fig. 1A)	ribbon-like, 1 mm wide	narrow ribbon-like or subcylindrical, compressed, 0.4–1.3 mm wide, 0.4–0.5 mm thick	subcylindrical, compressed, 0.5–1(–1.3) mm wide
Leaf	2–5 per tuft, linear-spatulate, flat, apex obtuse, 1.3–8.2 × 0.1–0.5 mm (Fig. 1B)	—	linear or subulate, apex acute or obtuse, 1.3–5 mm long	to 5 per tuft, linear, flat, apex obtuse, to 6 mm
Flowering shoot[*4]	3.5–5 mm long	1.4–2 mm long	3.5–10(–13) mm long	1.6–3(–3.5) mm long
Bract	10–18, upper bracts larger than lower ones, 1.5–2.5 × 3–3.5 mm, digitate (Fig.1C, D)	6–12, upper bracts equal to or slightly larger than lower ones	12–19(–36), upper bracts larger than lower ones, 1–3 × 0.7–2.8 mm, digitate	8–12(–14), all bracts subequal except smaller basal ones, digitate
Finger-like lobe	4–8, to 1.1 × 0.3 mm, longer than proximal lamina, subterete, rigid, rough	—	3–7, 0.3–1 mm long, median lobe linear-spatulate, terete, rigid, obtuse	3–9, to 0.7 × 0.3 mm, occasionally to 5 mm long, oblong or linear, rough
Spathella	globose, mucronate, mucro 0.4 mm long, ruptured near apex, funnel-like	—	globose, 1.3–1.9 × 0.9–1.4 mm, apex mucronate or forked mucronate, ruptured transversely around circumference on the upper side, lower part funnel-shaped	globose, apex mucronate, mucro simple or sometimes forked, ca. 0.4 mm, irregularly rupturing near apex
Pedicel	0.5–1.4 mm long	—	0.5–2 mm long	ca. 1.5 mm long
Tepal	2, filiform or linear-spatulate, 0.7–1.3 mm long	—	2, linear or subulate, 0.6–0.7 mm long, occasionally staminoid	2, linear, 1–1.5 mm long
Stamen	1, 1.7–2.3 mm long, as high as ovary or a little higher	—	1, ca. 1.3 mm long	1, to 1.5 mm long, as high as ovary
Anther	ca. 0.8 mm long, 4-thecate	—	ca. 0.9 mm × 0.8–0.9 mm, oblong elliptic	—
Pollen	dyad; (18–)19–21(–23) μm in diameter	—	two-celled (dyad)	—
Ovary	globose, unequally 2-locular, 1.3–1.7 × 1.1–1.2 mm	—	obliquely ovoid, smooth, unequally 2-locular, 1.1–1.5 × 0.7–1.3 mm	globose, 1–1.5 × 1 mm, 2-locular, with 2 nearly vertical grooves
Stigma	2, forked at base, procumbent, linear-obdeltoid, apex obliquely truncate, 0.6–0.9 mm long	—	2, forked at base, linear, apex acute or obtuse, 0.4–0.5 mm, deciduous	2, forked near base, procumbent, equal, linear to subulate, entire, ca. 0.6 mm long
Ovules	25–35 per locule, borne on entire surface of septum	—	25–35 per locule, borne on entire surface of septum	25–34 per locule, borne over surface of septum
Septum in ovary	convex in center, margin thin	—	convex in center, margin thin	convex in center, margin thin
Stalk of capsule	0.8–3 mm long	1.8–2.7 mm long	(0.5–)1.2–2.8 mm long	1.2–1.7 mm long
Capsule	globose, 1–1.5(–2.3) × 0.9–1.3 mm, smooth, 2-valved	globose, 1.1 × 1.0 mm, smooth	globose, 1.0–1.3 × 0.8–1.3 mm, smooth, 2-valved	globose, 1.5 mm long, smooth, 2-valved
Seed	0.2–0.3 × 0.15–0.2 mm	—	0.3–0.5 × 0.2–0.3 mm	—

[*1] The specimen comprises dried, more or less shrunk reproductive shoots with root segments.
[*2] Chao (1948), Kadono & Usui (1995) and present study.
[*3] *GK17125* is assigned to this species; Kato & Kita (2003) and present study.
[*4] The length from the base of reproductive shoot to the tip of uppermost bracts.

gata for providing material from Guangdong, and Y. Hirayama for technical assistance in the molecular phylogenetic analysis. We acknowledge the useful comments of two anonymous reviewers. We are also indebted to the directors of the University of Tokyo Herbarium (TI) and the National Museum of Nature and Science Herbarium (TNS), who allowed us to examine the specimens in their care. We are grateful to D. E. Boufford for the linguistic corrections. This study was supported by JSPS KAKENHI Grant Numbers 25291091 (to MK) and 26870502 (to SK).

References

Chao, H.C. 1948. Discovery of Podostemaceae in China. Contr. Inst. Bot. Natl. Acad. Peiping 6: 1–16.

Chao, H.C. 1980. A new genus (*Terniopsis* gen. nov.) of Podostemaceae from Fujian, China. Acta Bot. Yunnan. 2: 296–299.

Chao, H.C. 1982. Podostemaceae. *In*: Fl. Fukien, Vol. 1, pp. 479–482. (in Chinese with Latin synonyms)

Chun, W.Y. & Y. Tsiang. 1963. Materials for the Flora of Hainan (I). Acta Phytotax. Sin. 8: 259–278. (in Chinese)

Cusset, C. 1992. Contribution a l'étude des Podostemaceae: 12. Les genres asiatiques. Bull. Mus. Natl. Hist. Nat., B, Adansonia 14: 13–54.

Deng, Y.F. & N.H. Xia. 2008. Podostemaceae. *In*: Hong Kong Herbarium and South China Botanical Garden (eds.), Flora of Hong Kong, Vol. 2, pp. 122–123.

Kadono, Y. & N. Usui. 1995. *Cladopus austro-osumiensis* (Podostemaceae), a new rheophyte from Japan. Acta Phytotax. Geobot. 46: 131–135.

Kato, M. 2006. Taxonomic study of Podostemaceae of Thailand 2. Subfamily Podostemoideae with ribbon-like roots and subfamily Tristichoideae. Acta Phytotax. Geobot. 57: 1–54.

Kato, M. 2008. A taxonomic study of Podostemaceae of Japan. Bull. Natl. Mus. Nat. Sci., ser. B (Bot.) 34: 63–73.

Kato, M. 2013. The Illustrated Book of Plant Systematics in Color: Podostemaceae of the World. Hokuryukan, Tokyo.

Kato, M. 2016. Multidisciplinary studies of the diversity and evolution in river-weeds. J. Pl. Res. 129: 397–410.

Kato, M. & Y. Kita. 2003. Taxonomic study of Podostemaceae of China. Acta Phytotax. Geobot. 54: 87–97.

Kita, Y. & M. Kato. 2004. Molecular phylogeny of *Cladopus* and *Hydrobryum* (Podostemaceae, Podostemoideae) with implications for their biogeography in East Asia. Syst. Bot. 29: 921–932.

Koi, S. & M. Kato. 2010. Developmental morphology of seedling and shoot and phylogenetic relationship of *Diplobryum koyamae* (Podostemaceae). Amer. J. Bot. 97: 373–387.

Koi, S. & M. Kato. 2012. A taxonomic study of Podostemaceae subfamily Podostemoideae of Laos with phylogenetic analyses of *Cladopus, Paracladopus* and *Polypleurum*. Kew Bull. 67: 331–365.

Koi, S., Y. Kita, Y. Hirayama, R. Rutishauser, K. A. Huber & M. Kato. 2012. Molecular phylogenetic analysis of Podostemaceae: implications for taxonomy of major groups. Bot. J. Linn. Soc. 169: 461–492.

Koi, S., Y. Kita & M. Kato. 2008. *Paracladopus chanthaburiensis*, a new species of Podostemaceae from Thailand, with notes on its morphology, phylogeny and distribution. Taxon 57: 201–210.

Qiu, H., H.H. Chiu, H.X. Kiu & C. T. Philbrick. 2003. Podostemaceae. *In*: Wu, Z.Y. & P. H. Raven (eds.), Flora of China, Vol. 5, pp. 190–191.

Tao, D.D. 1983. *Hydrobryum* Endl.—a new recorded genus of Podostemaceae in China. Acta Bot. Yunnan. 5: 175–176. (in Chinese)

van Steenis, C. G. G. J. 1981. Rheophytes of the World. Sijthoff and Noordhoff, Alphen aan den Rijn, The Netherlands.

Wu, T.L. 1988. Podostemales. *In*: Fl. Reipubl. Popularis Sin., Vol. 24, pp. 1–5. (in Chinese)

Received April 9, 2016; accepted June 24, 2016